

Claims:

1. An isolated oligonucleotide not more than 60 nucleotides in length comprising:
 - (a) a nucleotide sequence of at least 10 contiguous nucleotides from a sequence
5 selected from the group consisting of SEQ ID NOS:1-16, 34, 35, 37, 42, 43, 45, 46, 50
and 52-55; (b) a nucleotide sequence having 90% sequence identity to a nucleotide
sequence of (a); or
 - (c) complements of (a) and (b).
- 10 2. The oligonucleotide of claim 1, wherein the oligonucleotide is a nucleotide
sequence of at least 10 contiguous nucleotides from a sequence selected from the group
consisting of SEQ ID NOS: 1-16, 34, 35, 37, 42, 43, 45, 46, 50 and 52-55.
- 15 3. The oligonucleotide of claim 1, selected from the group consisting of SEQ ID
NOS:1-16, 45, 46 and 50.
4. The oligonucleotide of claim 2, selected from the group consisting of SEQ ID
NOS:1-16, 45, 46 and 50.
- 20 5. The oligonucleotide of claim 1, selected from the group consisting of SEQ ID
NOS:34, 35, 37, 38, 42 and 43.
6. The oligonucleotide of claim 2, selected from the group consisting of SEQ ID
NOS:34, 35, 37, 38, 42 and 43.
- 25 7. A labeled oligonucleotide not more than 60 nucleotides in length comprising:
 - (a) a nucleotide sequence of at least 10 contiguous nucleotides from a sequence
selected from the group consisting of SEQ ID NOS:52, 53, 54 and 55; (b) a nucleotide
sequence having 90% sequence identity to a nucleotide sequence of (a); or

(c) complements of (a) and (b).

8. The oligonucleotide of claim 7, wherein the oligonucleotide is a nucleotide sequence of at least 10 contiguous nucleotides from a sequence selected from the group consisting of SEQ ID NOS: 52, 53, 54 and 55.

9. The oligonucleotide of either of claims 7 or 8, further comprising a detectable label at the 5'-end and/or the 3'-end.

10. The oligonucleotide of claim 9, wherein the detectable label is a fluorescent label selected from the group consisting of 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7', - tetrachloro -4-7- dichlorofluorescein (TET).

11. The oligonucleotide of claim 10, wherein the oligonucleotide is selected from the group consisting SEQ ID NOS:36, 39, 44 and 45.

12. A method for detecting the presence of West Nile virus (WNV) in a biological sample, the method comprising:

isolating nucleic acids from a biological sample suspected of containing WNV;
amplifying the nucleic acids using a sense and an antisense primer wherein each of the primers is not more than about 60 nucleotides in length and is sufficiently complementary to a portion of the sense and antisense strands, respectively, of the isolated nucleic acid to hybridize therewith, and

(a) the sense primer comprises SEQ ID NO:34 or a nucleotide sequence having at least 90% sequence identity thereto, or SEQ ID NO:37 or a nucleotide sequence having at least 90% sequence identity thereto, or SEQ ID NO:42 or a nucleotide sequence having at least 90% sequence identity thereto;

(b) the antisense primer comprises SEQ ID NO:35 or a nucleotide sequence having at least 90% sequence identity thereto when the sense primer is SEQ ID NO:34, or

the antisense primer comprises SEQ ID NO:38 or a nucleotide sequence having at least 90% sequence identity thereto when the sense primer is SEQ ID NO:37, or the antisense primer comprises SEQ ID NO:43 or a nucleotide sequence having at least 90% sequence identity thereto when the sense primer is SEQ ID NO:42; and

- 5 detecting the presence of the amplified nucleic acids as an indication of the presence of WNV in the sample.

13. The method of claim 12, wherein the nucleic acids are isolated from the biological sample by a method comprising:

- 10 (a) contacting a solid support comprising capture nucleic acids associated therewith with a biological sample under hybridizing conditions wherein WNV nucleic acid strands, if present in the biological sample, hybridize with the capture nucleic acids; and

 (b) separating the solid support from the sample.

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14. The method of claim 13, wherein the solid support comprises beads.

15. The method of claim 14, wherein the beads are magnetic beads.

- 20 16. The method of claim 15, wherein the isolating, amplifying and detecting are performed in a single container.

17. The method of claim 13, wherein the capture nucleic acids comprise one or more oligonucleotides, wherein each of the oligonucleotides is not more than about 60
25 nucleotides in length and comprises at least 10 contiguous nucleotides from a sequence selected from the group consisting of SEQ ID NOS:1-16, 45, 46 and 50.

18. The method of claim 17, wherein the capture nucleic acids further comprise a homopolymer chain of about 10-25 nucleotides in length, selected from the group

consisting of polyA, polyT, polyG, polyC, and polyU.

19. The method of claim 18, wherein the homopolymer chain is a polyA chain.

5 20. The method of claim 12, wherein amplifying comprises RT-PCR, transcription-mediated amplification (TMA) or TaqManTM, or a combination thereof.

 21. The method of claim 13, wherein amplifying comprises TaqManTM using the sense primer and the antisense primer and detecting is done using at least one probe
10 comprising a detectable label.

 22. The method of claim 21, wherein the at least one probe is not more than 60 nucleotides in length and comprises (a) the sequence of SEQ ID NO:52 or the sequence of SEQ ID NO:53 when the sense primer comprises the sequence of SEQ ID NO:34 or
15 (b) the sequence of SEQ ID NO:54 when the sense primer comprises the sequence of SEQ ID NO:37 or (c) the sequence of SEQ ID NO:55 when the sense primer comprises the sequence of SEQ ID NO:42.

 23. The method of claim 22, wherein the method comprises using a probe
20 comprising the sequence of SEQ ID NO:52 and a probe comprising the sequence of SEQ ID NO:53 when the sense primer comprises the sequence of SEQ ID NO:34.

 24. The method of either of claims 22 or 23, wherein the probe further comprises detectable labels at the 5'-end and at the 3'-end.

25 25. The method of claim 21, wherein the detectable label is a fluorescent label selected from the group consisting of 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7',- tetrachloro -4-7- dichlorofluorescein (TET).

26. The method of claim 12, wherein an internal control sequence is present.

27. The method of claim 26, wherein the internal control sequence comprises the nucleotide sequence of Figure 2 (SEQ ID NO:17).

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28. The method of claim 27, further comprising a detectably labeled probe sequence for the internal control sequence.

29. The method of claim 28, wherein the detectably labeled probe sequence for
10 the internal control sequence comprises the sequence of SEQ ID NO:40 or SEQ ID NO:41.

30. A kit for detecting the presence of West Nile virus (WNV) in a biological sample, the kit comprising:

15 capture nucleic acids comprising one or more oligonucleotides, wherein each of the oligonucleotides is not more than about 60 nucleotides in length and comprises a nucleotide sequence of at least 10 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS:1-16, 45, 46 and 50;

primer oligonucleotides wherein the primer oligonucleotides are not more than
20 about 60 nucleotides in length and comprise a nucleotide sequence of at least 10 contiguous nucleotides from SEQ ID NOS:34 and 35 or SEQ ID NOS:37 and 38 or SEQ ID NOS:42 and 43; and

written instructions for identifying the presence of WNV.

25 31. The kit of claim 30, further comprising a polymerase and buffers.

32. The kit of claim 30, further comprising at least one probe oligonucleotide of not more than about 60 nucleotides in length and at least 10 contiguous nucleotides, wherein the at least one probe oligonucleotide comprises (a) the sequence of SEQ ID

NO:52 or the sequence of SEQ ID NO:53 when the primer oligonucleotides comprise at least 10 contiguous nucleotides from SEQ ID NO:34 and SEQ ID NO:35; or (b) the sequence of SEQ ID NO:54 when the primer oligonucleotides comprise at least 10 contiguous nucleotides from SEQ ID NO:37 and SEQ ID NO:38; or (c) the sequence of
5 SEQ ID NO:55 when the primer oligonucleotides comprise at least 10 contiguous nucleotides from SEQ ID NO:42 and SEQ ID NO:43.

33. The kit of claim 32, wherein the probe further comprises detectable labels at the 5'-end and at the 3'-end.
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34. The kit of claim 33, wherein the detectable label is a fluorescent label selected from the group consisting of 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7',- tetrachloro -4-7- dichlorofluorescein (TET).

15 35. The kit of claim 32, wherein the kit comprises a probe comprising the sequence of SEQ ID NO:36 and a probe comprising the sequence of SEQ ID NO:49 when the sense primer comprises the sequence of SEQ ID NO:34.

20 36. The kit of claim 32, further comprising an internal control comprising the nucleotide sequence of Figure 2 (SEQ ID NO:17).

25 37. A pair of amplification primers for detecting WNV comprising a pair of oligonucleotides selected from the group consisting of the SEQ ID NO:34/SEQ ID NO:35 pair, the SEQ ID NO:37/SEQ ID NO:38 pair and the SEQ ID NO:42/SEQ ID NO:43 pair.

38. A set of oligonucleotides for specifically capturing WNV nucleic acid comprising an oligonucleotide of no more than 60 nucleotides in length and comprising the sequence SEQ ID NO:8, an oligonucleotide of no more than 60 nucleotides in length

and comprising the sequence SEQ ID NO:12, an oligonucleotide of no more than 60 nucleotides in length and comprising the sequence SEQ ID NO:45, and an oligonucleotide of no more than 60 nucleotides in length and comprising the sequence SEQ ID NO:46.

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39. The set of oligonucleotides of claim 38, further comprising an oligonucleotide of no more than 60 nucleotides in length and comprising the sequence SEQ ID NO:50.

40. A method of preparing a blood supply comprising whole blood, plasma or serum, substantially free of West Nile Virus (WNV) comprising:

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(a) screening aliquots of whole blood, plasma or serum from collected blood samples by the method of claim 12;

(b) eliminating samples where WNV is detected; and

(c) combining samples where WNV is not detected to provide a blood supply substantially free of WNV.

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